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RESEARCH ARTICLE

PHYTOCHEMISTRY OF THREE INDIAN VARIETIES OF *PUNICAGRANATUM* AND VITAMIN-C STUDY BY HPLC TECHNIQUE

Pavan C. Akkiraju^{1*}, Harshad S. Tambe¹, Dipeeka D. Suryawanshi¹, Srilakshmi Mamillapalli² and Aparna J. Jawakekar¹.

1. Department of Biotechnology, P.V.P. College of Arts, Science & Commerce, Pravaranagar, Loni, Ahmednagar (Dt.), Maharashtra.
2. Department of Pharmacy, Sarada College of Pharmaceutical Sciences, Kondakavuru, Narasaraopet, Guntur, (Dt.), A.P., India.

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Abstract

Three Indian pomegranate varieties with different physiological properties have been studied for their phytochemistry and ascorbic acid content. Local varieties of *Punicagranatum* L. namely *Bhagwa*, *Ganesh* and *Mridula* were selected for analysis. The three parts of pomegranate viz. exocarp, mesocarp and seeds were separately processed as methanol extractions for various phytochemicals and HPLC analysis for Vitamin-C content. Flavonoids were found absent in the seeds of three varieties, whereas they were present in the exocarp and mesocarp of the same. On other hand, tannins were present in the seeds and absent in the exocarp and mesocarp of all varieties. Phycobilins were absent in all parts of *Ganesh* and mesocarp of *Mridula*. Out of all varieties, the mesocarp of *Ganesh* has high content of ascorbic acid with an area of 90.14% with a TP content of 4784.9µg/mg and TF content of 1.6364µg/mg. Out of three varieties, exocarp, mesocarp and seeds of *Ganesh* resulted with more ascorbic acid content.

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Introduction:-

A multitude of organic molecules or phytochemicals, generally referred as secondary metabolites are synthesized by vascular plants. These phenolic compounds are synthesized during plant development and are plays vital roles in the plant life cycle (Stalikas, 2007). Based on metabolic utilization, the plant produces primary constituents which include sugars, proteins and chlorophyll, whereas secondary constituents include alkaloids, steroids, terpenoids, etc. (Dhawale, 2013). Various fruits and vegetables have been confirmed for their phytochemical properties which include oranges and green fruits and vegetables (carotenoids); Apples, citrus fruits, onions, soybeans and products (flavonoids); Green tea, wine, grapes, berries, whole grains and peanuts (Polyphenols); Cherries, citrus fruits (Terpenes). *Punicagranatum* is a fruit, highly grows in Mediterranean regions under drought conditions (Chakraborty Manodeepet *et al.* 2012). Pomegranates also showed many medicinal properties ranging from reduced oxidative stress, platelet aggregation (Aviram *et al.* 2000), antioxidant and antibacterial (Negi and Jayaprakasha, 2003) and antiviral (Zhang *et al.* 1995) activities.

Pomegranate can be separated into three major parts: peel, which includes exocarp, mesocarp and the edible portion, Seeds. The peel of pomegranates contains good levels of phenolic compounds including tannins and flavonoids

Corresponding Author:-Pavan C. Akkiraju.

Address:-Department of Biotechnology, P.V.P. College of Arts, Science & Commerce, Pravaranagar, Loni, Ahmednagar (Dt.), Maharashtra.

(Zhao *et al.* 2014). The edible plant parts are juice, pulp and seeds, which constitute around 65-75% of total fruit weight (Tehrani *et al.*, 2010). The taste of the juice and seeds vary among different varieties of pomegranate. The taste may be sweet, sour or sweet-sour. However, these portions are rich in sugars, minerals and various phenolic constituents (Mansour *et al.* 2014).

The present study aimed to study various phytochemical compounds present in the exocarp, mesocarp and seeds of three varieties of pomegranates: *Bhagwa*, *Ganesh* and *Mridula*. The main objectives of the study include to identify the phytochemical constituents present in all the parts of the three varieties and to analyze ascorbic acid content from the same.

Materials & Methods:-

Twenty individual fruits from each variety were collected randomly from the local markets of Loni, Maharashtra separately to study the phytochemistry. *Bhagwa* is a fruit with orange-red fruit having peach-red colored seeds with sweet taste. *Ganesh* is a yellowish fruit with soft and pink colored seeds having sour-sweet taste. *Mridula* is a red fruit with red seeds and sour-sweet taste (Fig. 1).

The fruits were collected separately in wooden boxes and stored at the Laboratory of Biotechnology, P.V.P. College, Loni. The exocarp, mesocarp and seeds were isolated manually and they were dried in hot air oven at 50°C until they dried completely. The dried plant materials were grinded by using a house-hold mixer grinder. The Soxhlet procedure for methanol extraction is performed (Akkiraju *et al.* 2016) and collected the extracted powder separately from each part of three varieties of pomegranates (Fig. 1). These powders are then subjected to various studies of primary Phytochemical compounds viz. test for saponins, proteins, carbohydrates, amino acids, glycosides and secondary Phytochemical compounds viz. test for alkaloids, flavonoids, vitamin-C, anthraquinones, phycobilins and tannins. The presence or absence of the mentioned compounds and HPLC analysis for ascorbic acid were performed according to Akkiraju *et al.* (2016).

Results:-

The triplicates of tests performed for the identification of different phytochemicals was tabulated (**Table 1**). The saponins, alkaloid, carbohydrates and ascorbic acid were found present in all the parts of *Bhagwa*, *Ganesh* and *Mridula*. Glycosides, proteins, amino acids and anthraquinones are absent in all the parts of all three varieties. The flavonoids were present in exocarp and mesocarp of three varieties, but found absent in the seeds. The tannins were absent in the exocarp and mesocarp of all three varieties and resulted positive in seeds. Phycobilins were positive in all the parts of *Bhagwa* and negative in all parts of *Ganesh*. In *Mridula*, exocarp and seeds have phycobilins, but mesocarp does not possess them.

Ascorbic acid analysis by HPLC was performed and the results were obtained (**Fig. 2a, b, c; Table 2**). The same has revealed ascorbic acid peaks and compared with the standard. The chromatogram for the exocarp, mesocarp and seeds showed peaks confirming the quantity of Vitamin-C. The mesocarp of *Ganesh* has showed the peak within a retention time of 3.9167 min, whereas the exocarp of *Mridula* showed the highest RT (4.3833 min.). The maximum peak area was identified for the exocarp of *Mridula* with an area of 84335.227 mV*s with an area% of 81.79%, where it showed a significant TP value of 456.1 and TF value of 0.5469. The least area peak area was observed for the seeds of *Ganesh* with an area of 1144.852 mV*s with an area% of 40.72%. In respect to area%, Mesocarp has a maximum area% of 90.14% and minimum was for the seeds of *Mridula* with 30.17%. The maximum number of Theoretical Plates (TP) was observed for the mesocarp of *Ganesh* with 4784.9 and the minimum was found in the exocarp of *Mridula* with a TP of 456.1. The exocarp of *Ganesh* has a maximum TF value (1.75) and the same of *Mridula* has a minimum TF value (0.5469).

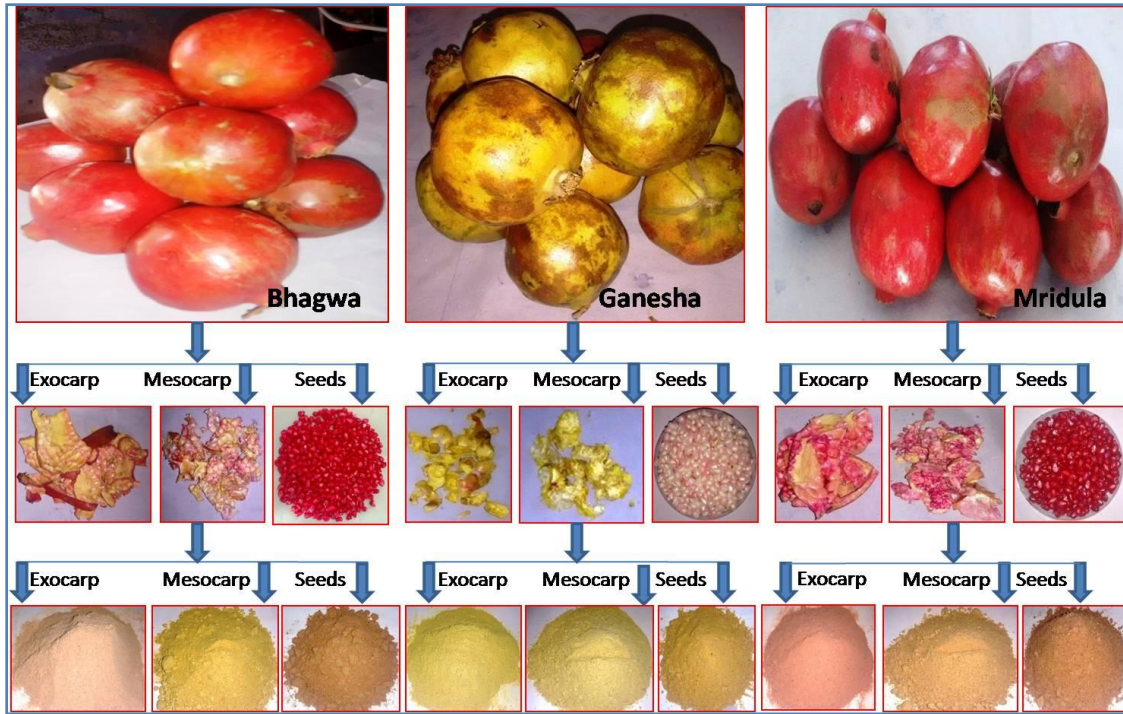


Figure 1:- Preparation of powders from exocarp, mesocarp and seeds of *Bhagwa*, *Ganesha* and *Mridula*.

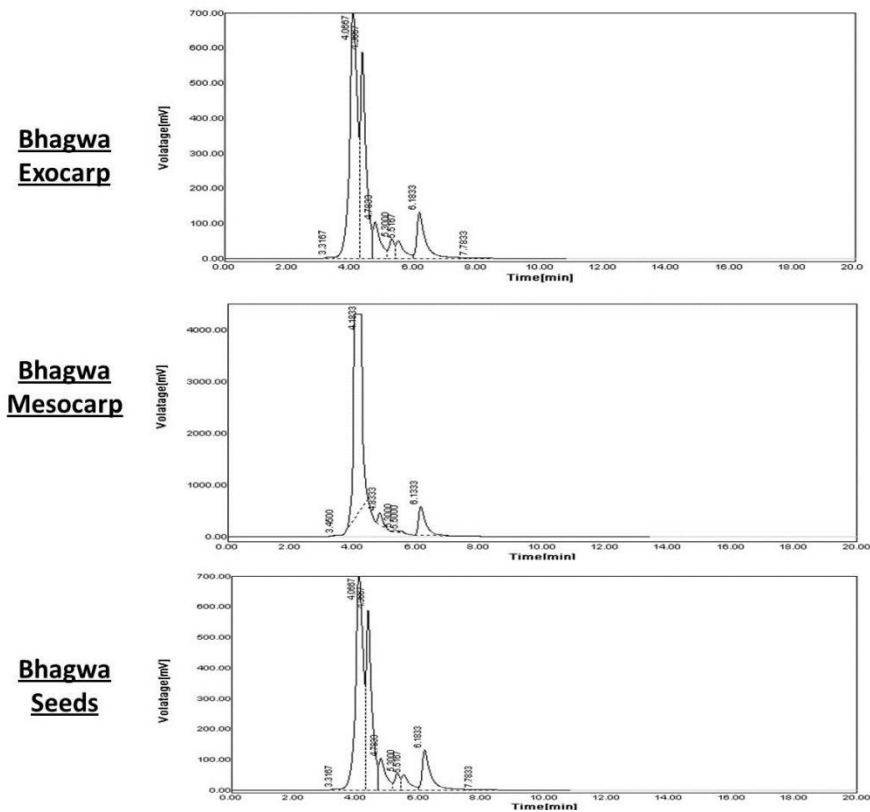
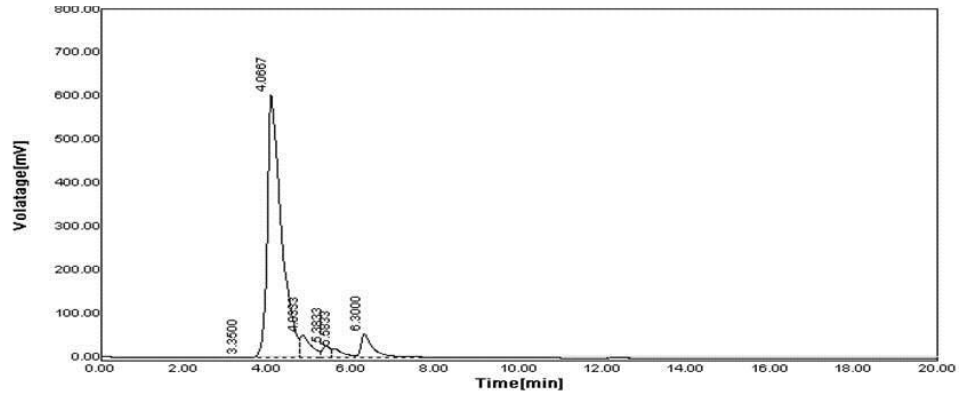
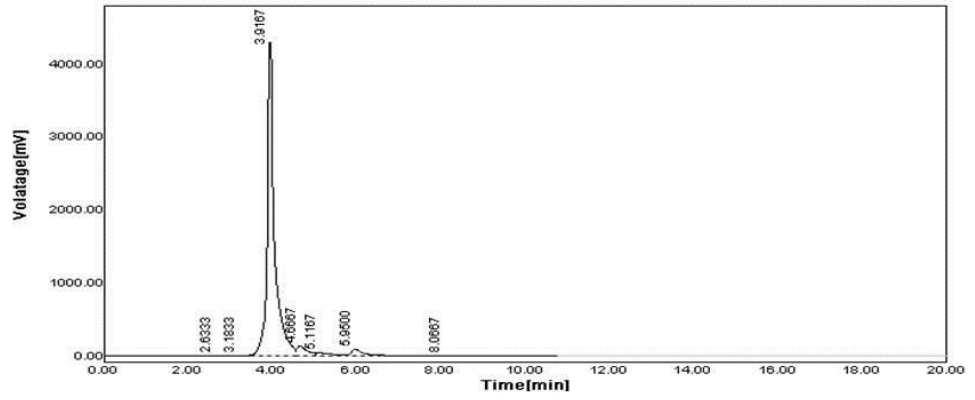


Figure 2 (a):-Ascorbic acid analysis by using HPLC in *Bhagwa* exocarp, mesocarp and seeds.

Ganesha
Exocarp



Ganesha
Mesocarp



Ganesha
Seeds

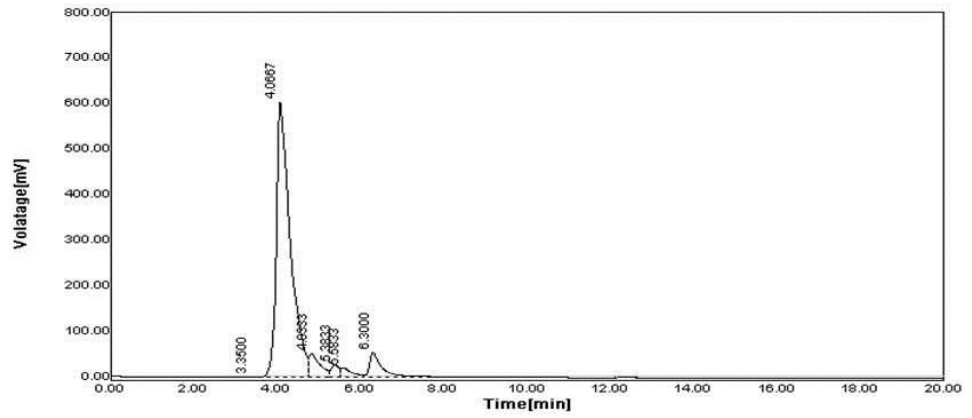


Figure 2 (b):- Ascorbic acid analysis by using HPLC in *Ganesha* exocarp, mesocarp and seeds.

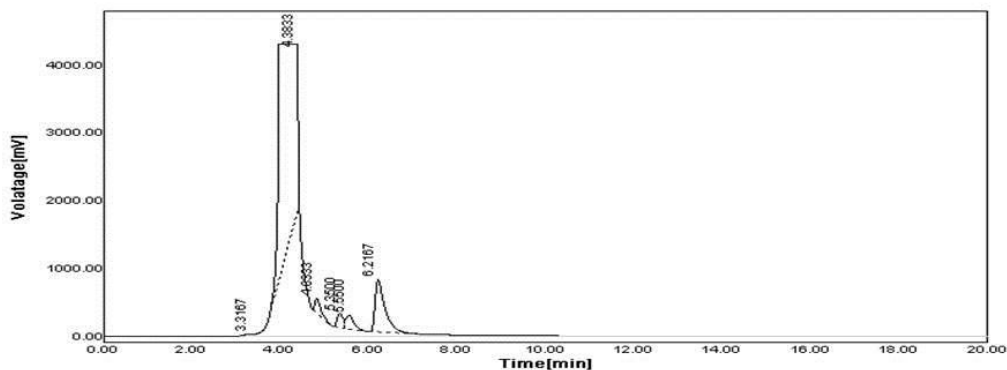
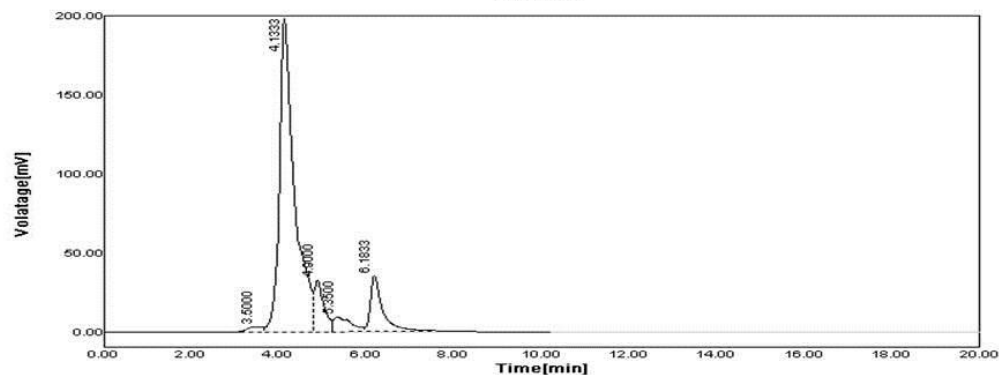
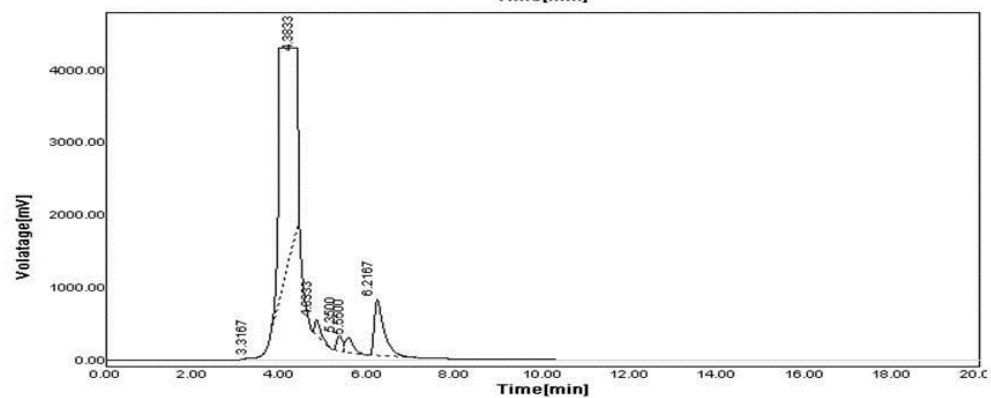
Mridula
Exocarp**Mridula**
Mesocarp**Mridula**
Seeds

Figure 2 (c):-Ascorbic acid analysis by using HPLC in *Mridula* exocarp, mesocarp and seeds.

Table 1:-List of Phytochemicals tested and their presence or absence in different parts of pomegranates.

S. No.	Test Compound	Bhagwa			Ganesha			Mridula		
		Exo Carp	Meso Carp	Seeds	Exo carp	Meso carp	Seeds	Exo carp	Meso carp	Seeds
1	Saponins	+	+	+	+	+	+	+	+	+
2	Flavonoids	+	+	-	+	+	-	+	+	-
3	Glycosides	-	-	-	-	-	-	-	-	-
4	Proteins	-	-	-	-	-	-	-	-	-
5	Carbohydrates	+	+	+	+	+	+	+	+	+
6	Alkaloids	+	+	+	+	+	+	+	+	+
7	Amino acids	-	-	-	-	-	-	-	-	-
8	Tannins	-	-	+	-	-	+	-	-	+
9	Anthraquinones	-	-	-	-	-	-	-	-	-
10	Phycobilins	+	+	+	-	-	-	+	-	+
11	Ascorbic acid	+	+	+	+	+	+	+	+	+

Table 2:-Ascorbic acid analysis: RT- Retention time; TP- Theoretical Plates; TF- Tailing Factor

	Name	RT[min]	Area[mV*s]	Area%	TP	TF
Exocarp	Bhagwa	4.0667	12075.139	47.28	1018.9	1.2500
	Ganesha	4.0667	14123.877	82.83	825.3	1.7500
	Mridula	4.3833	84335.227	81.79	456.1	0.5469
Mesocarp	Bhagwa	4.1833	75550.063	86.17	967.7	0.8947
	Ganesha	3.9167	52225.969	90.14	4784.9	1.6364
	Mridula	4.1333	4745.440	75.52	944.7	1.5000
Seeds	Bhagwa	4.1667	2462.467	33.71	475.4	1.0000
	Ganesha	4.1500	1144.852	40.72	596.9	1.0000
	Mridula	4.1833	3911.646	30.17	479.2	1.0909

Discussion:-

Evaluation of total phenolic contents, antioxidant activities and presence of vitamins in pomegranate were previously reported and discussed. Elfalleh *et al.* (2012) showed that the methanolic extracts to study alkaloids, flavonoids and tannins were suitable for phytochemical screening, which is supported by this current study. However, we found the difference among three varieties of pomegranates in respect to flavonoids and tannins. Alkaloids were present in all the varieties and studied through methanol extractions. We found that flavonoids and tannins were oppositely present among the varieties. In all the varieties, exocarp and mesocarp contained flavonoids, but seeds contained only tannins. Bhandari *et al.* (2012), while performing the phytochemical screening with peel and seeds, revealed the presence of glycosides in peel (Chloroform) and seeds (Ethanol). However, in the current study, we found no glycoside content in any part of any variety. This may be due to methanol extraction. The same authors reported the absence of flavonoids in seeds, which is supported by our study. In respect to saponins and alkaloids, we observed them in all varieties and their parts, where as, these authors were not able to find the same in peels. These authors reported tannins in peels, where as we found tannins only in seeds. Chebaibiet *al.* (2013) showed the absence of alkaloids in peel extracts, where as our results showed alkaloids presence in the peel extracts. They found tannins and glycosides in peels, where as we found absence of the both in peels.

Anahita *et al.* (2015) evaluated antioxidant vitamin i.e. ascorbic acid from pomegranate juice and seed and concluded that a combination of both seed-juice have high phenolic compounds and vitamin-C. In the current study, the mesocarp of *Ganesha* showed high content of ascorbic acid (90.14%). It indicates that, mesocarp can be a complementary source for vitamin-c supplement.

Conclusion:-

The presence of various phytochemicals in pomegranate can promote the essential constituents in alimentary purposes. We found variation in phytochemical constituents among different varieties of pomegranates available in India. We found that the seeds of these varieties does not contain flavonoids, but are rich of tannins. These varieties showed absence of glycosides. Neither a single variety nor any part of these varieties showed glycoside's presence. We also found alkaloids in seeds. In respect to ascorbic acid, out of all the parts and varieties, the mesocarp of *Ganesha* showed high content of ascorbic acid.

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